Practical Endocrinology

WBC counting

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- White cell count ✹important component of the blood count✹ **white** in color, have a **nucleus** & Their function is **defense**
- White cells can be counted either **manually** or **automatically**.
- **Anticoagulated** venous blood is added to a **diluent** at a specific volume.
- The **diluent lyses** the erythrocytes, but **preserves** leukocytes.
- The diluted blood is added to the **hematocytometer** chamber.
- The diluent used is **2% acetic acid +gentian violet.**

Principle of WBCs count test

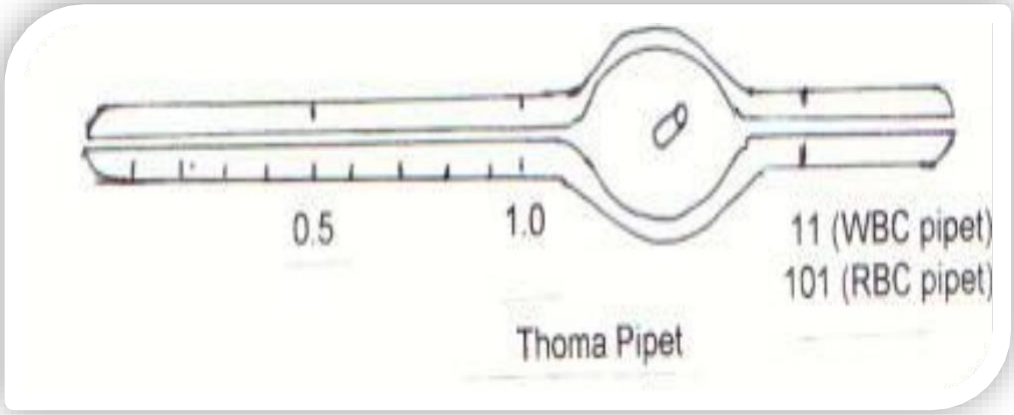
The diluent Türk's solution is

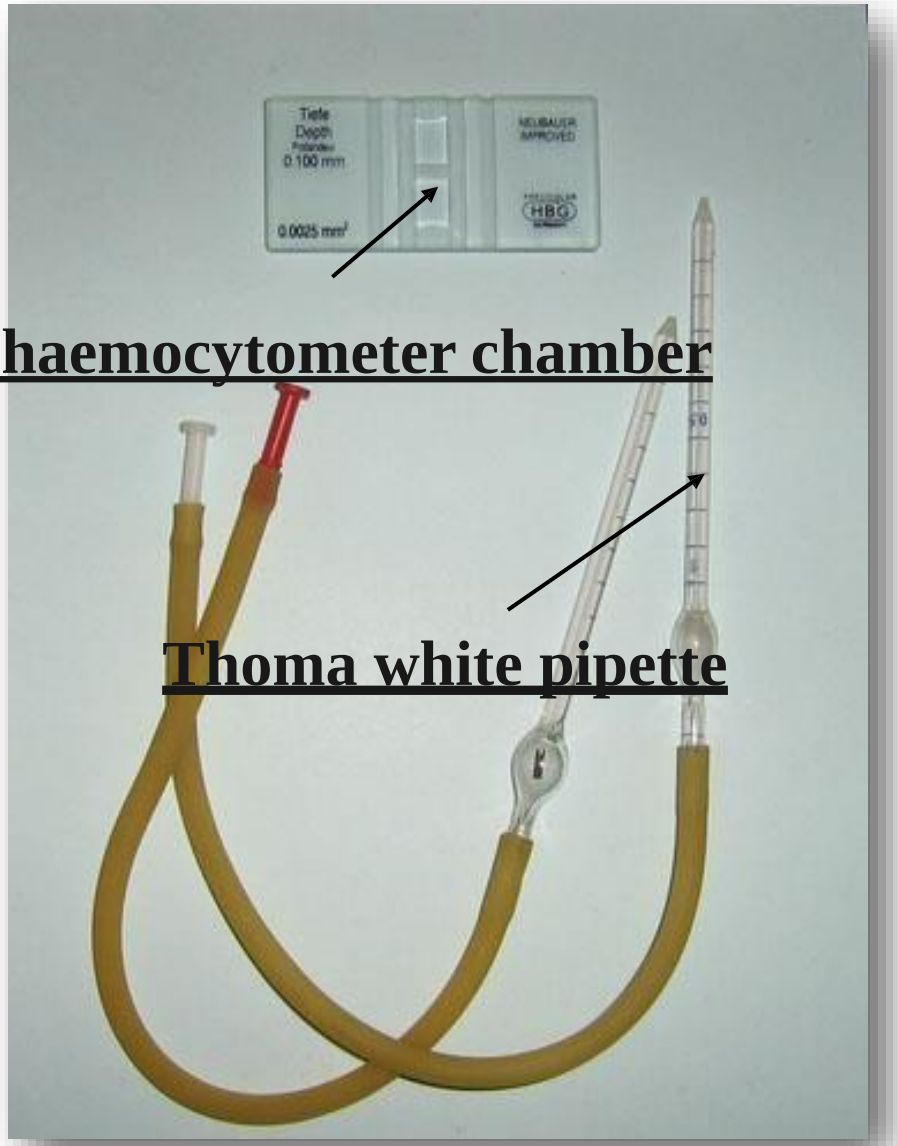
1. a hematological stain (crystal violet (gentian violet) or aqueous methylene blue) for staining the nuclei of the white blood cells, making them easier to see and count.

2. **in 1-2% acetic acid and distilled water**. The solution destroys the RBCs and platelets within a blood sample.

Equipment

1. White blood cells count diluting **fluid**
2. Thoma white **pipette**
3. **Hemacytometer** and coverslip
4. **Microscope**
5. Lint-free **wipe**
6. **Alcohol** pads





haemocytometer chamber

Thoma white pipette

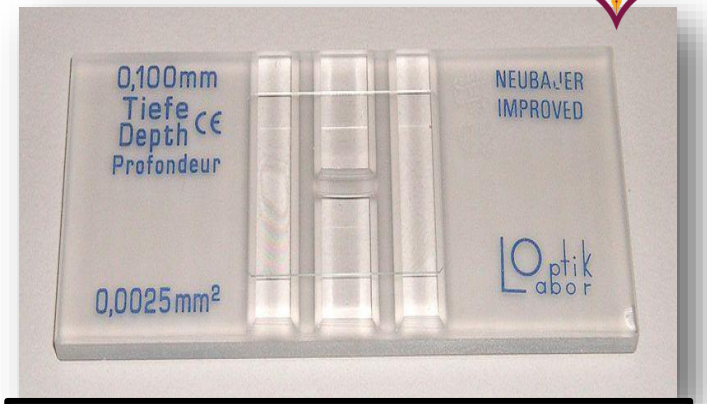
Rubber sucking tube

Requirements

White blood cells count diluting fluid

Microscope

Thoma white pipette



Hemacytometer and coverslip

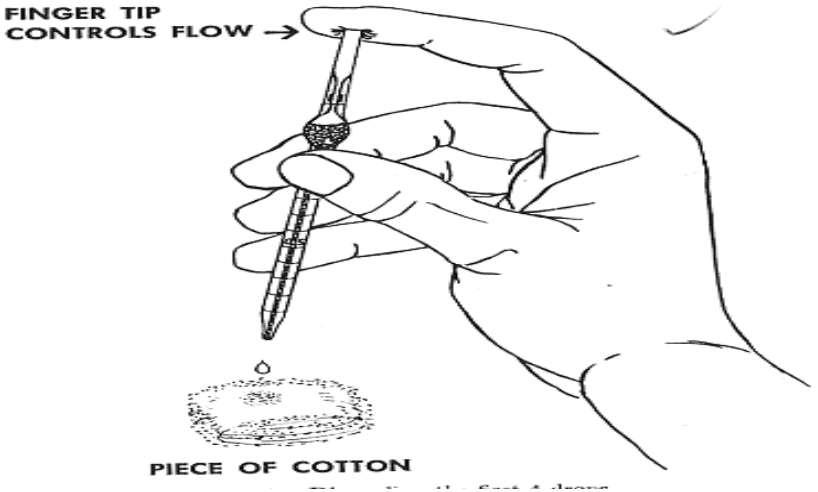
Alcohol pads

Procedure

1. Draw the blood up to 0.5 mark in the thoma pipette.
2. Wipe the outside of the capillary pipette to remove excess blood that would interfere with the dilution factor.
3. Holding the pipette almost vertical place into the fluid. Draw the diluting fluid into the pipette slowly until the mixture reaches the 11 mark, while gently rotating the pipette to ensure a proper amount of mixing.
4. Place the pipette in a horizontal position and firmly hold the index finger of either hand over the opening in the tip of the pipette, detach the aspirator from the other end of the pipette now the dilution of the blood is completed

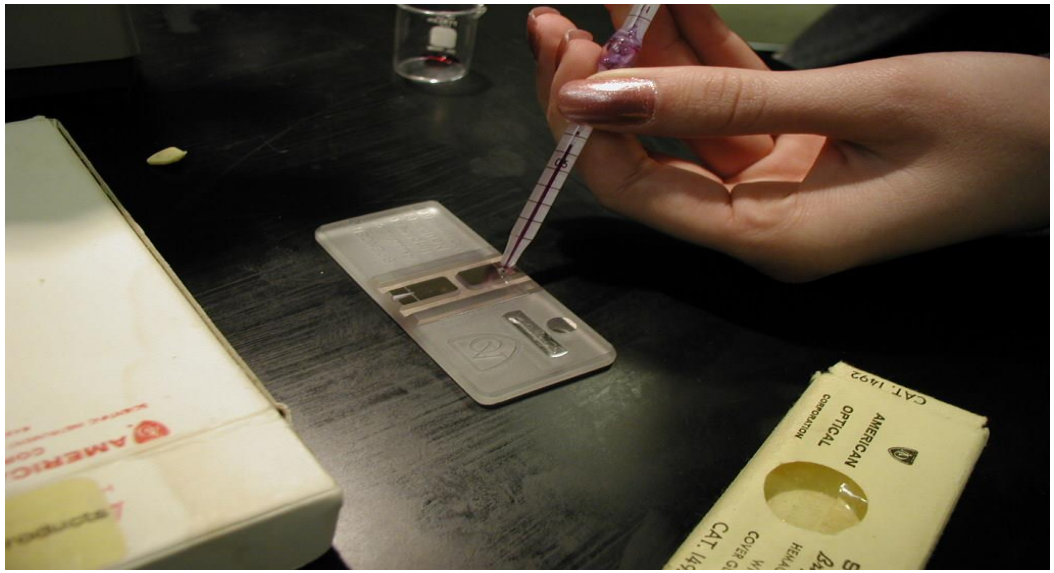
Procedure

5. Mix the sample for at least 3 minutes to facilitate hemolysis of RBCs.
6. Clean the hemacytometer and its coverslip with an alcohol pad and then dry with a wipe.
7. Before filling the chamber, discard the first four to five drops of the mixture on apiece of gauze to expel the diluent from the stem.



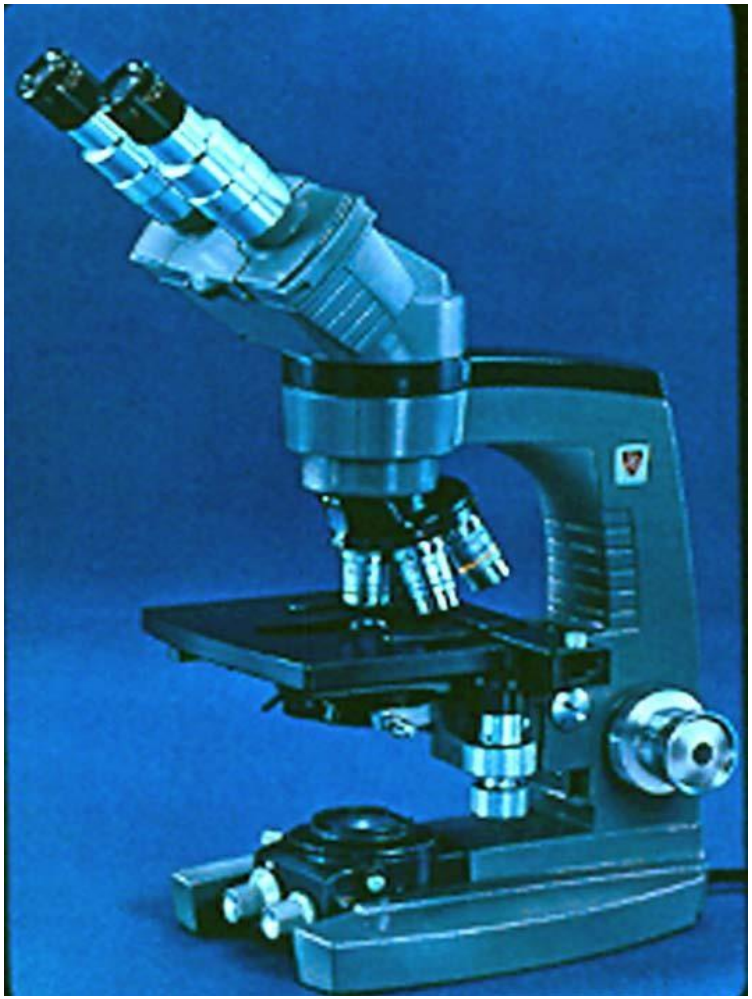
Procedure

8. Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.



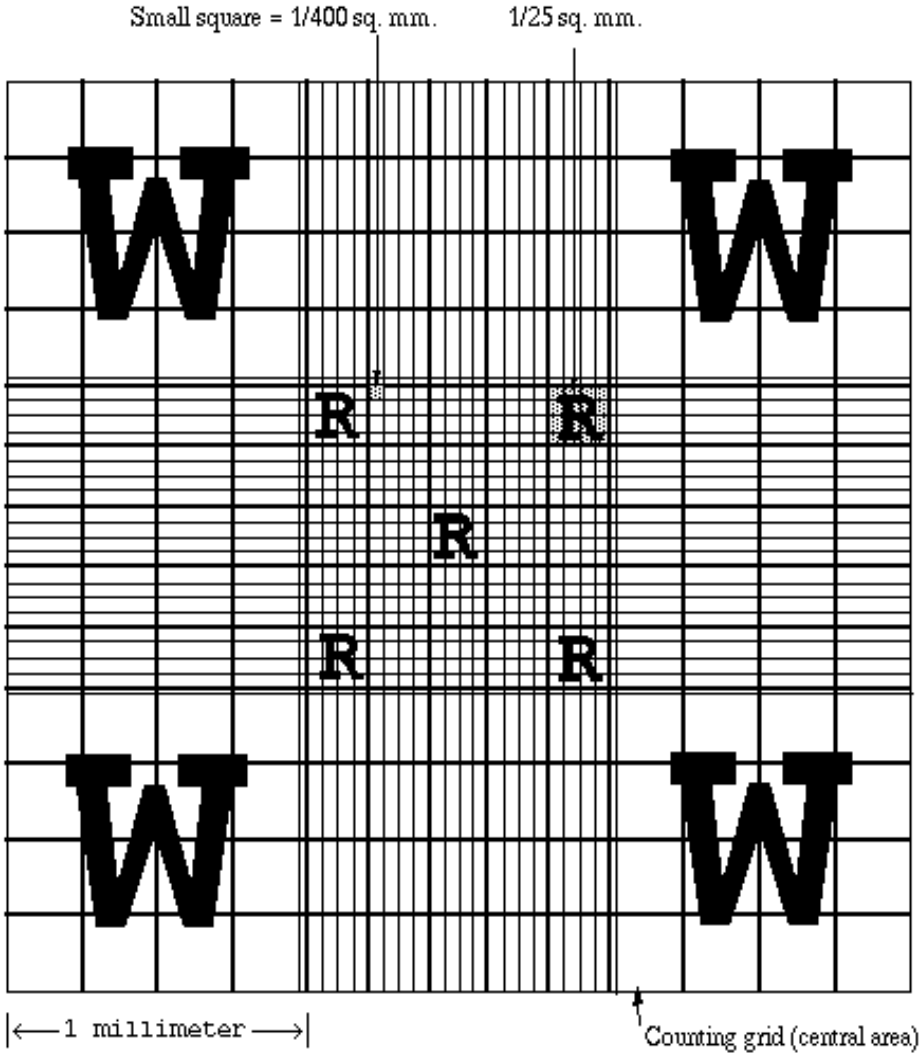
FOCUSING

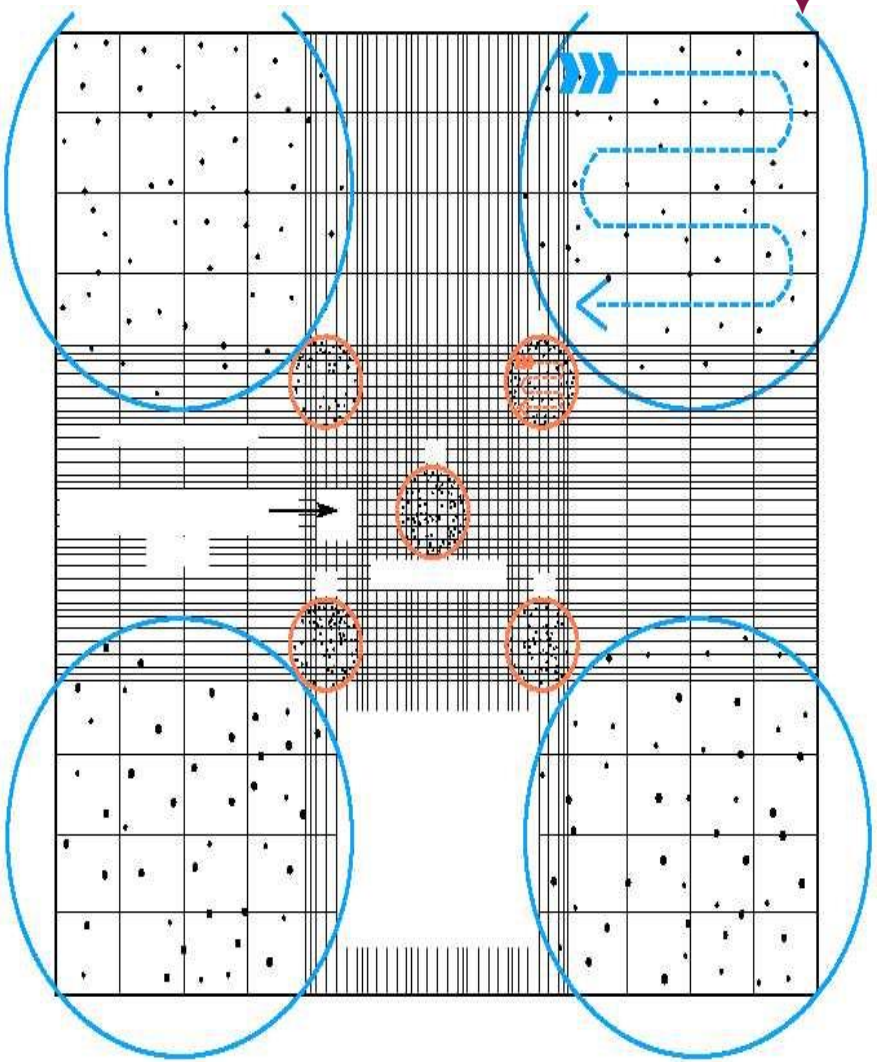
- 4X to see the general formation of slide.
- 10X for WBC counting
- 40X for RBC counting



Procedure for counting WBC's

1. Under 10 x magnifications, scan to ensure even distribution. Leukocytes are counted in all nine large squares of the counting chamber.
2. Count cells starting in the upper left large corner square. Move to the upper right corner square, bottom right corner square, and bottom left corner square.
3. Count all cells that touch any of the upper and left lines, do not count any cell that touches a lower or right line.


Small square = 1/400 sq. mm.
1/25 sq. mm.
W
R
1 millimeter
Counting grid (central area)



Total WBC Count per cu.mm (uL)

Calculation

WBC Count =

$$\frac{\text{No. of cells counted x Dilution factor x Depth factor}}{\text{Area counted}}$$

* Dilution factor: 20
* Depth factor: 10
* Area counted: 1sq.mm x 4 = 4 sq.mm
* No. of cells counted: "N"

Total WBC count per cu.mm (uL)

Shortcut method

WBC count =

Sum of No. of cells in all 4 squares
x 50

Sources of errors

Common Sources of Error:

a) Failure to have required blood volume.

b) Failure to mix well.

c) Failure to discard the first 4 drops.

d) Failure to properly charge the counting chamber.

The least Frequent Sources of Error are:

a) **Inaccurate** pipette or counting chamber.

b) **Moist** or **unclean** pipette.

c) **Excessive pressure** in the finger when obtaining the blood.

d) Too **little** or too **much** diluting fluid.

e) **Slowness** in manipulation, thus allowing the blood to clot.

f) Air **bubbles** in the pipette.

g) Air **bubbles** in the counting chamber.

h) Presence of **yeast** or other contaminants in the diluting fluid.

i) Mistakes in **counting or calculations**.

- Falsely high WBC count
  - Nucleated red cells
  - Non-lysis of red cells (due to target cells in hemoglobinopathy)
  - Giant platelets or platelet clumps (due to EDTA)
  - Cryoglobulins
  - Microorganisms

- Falsely low WBC count
  - Leukoagglutination (due to EDTA)
  - Cold agglutinin

Leukopenia

- HIV
- autoimmune disorders
- bone marrow disorders or damage
- lymphoma
- severe infections
- liver and spleen diseases
- lupus
- radiation therapy
- some medications, such as antibiotics

Leukocytosis

- smoking
- infections such as tuberculosis
- tumors in the bone marrow
- leukemia
- inflammatory conditions, such as arthritis and bowel disease
- stress
- exercise
- tissue damage
- pregnancy
- allergies
- asthma
- some medications, such as corticosteroids

Age	Normal WBC Count (cells/mm^3 of blood)
At birth	10,000-25,000
Infant	8,000-15,000
4-7 y	6,000-15,000
8-18 y	4,500-13,500
Adults	4000-10000
Pregnancy	12,000 to 15,000